*Reference*: *Biol. Bull.*, 152: 105–119. (February, 1977)

# CYTOCHEMICAL ASPECTS OF *MERCENARIA MERCENARIA* HEMOCYTES

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Much work has been published on the cytology of molluscan hemocytes. Cuénot (1891), working with several lamellibranchs, recognized the existence of three cell types; Drew and Cantab (1910) also classified hemocytes of *Cardium norvegicum* into three morphological classes. Takatsuki (1934) described two hemocyte types in *Ostrea edulis*.

Zacks and Welch (1953) demonstrated cholinesterase and lipase in hemocytes of *Mercenaria mercenaria*, and Zacks (1955) published the first extensive cytological and cytochemical study on the blood cells of this animal. Only one type of cell was described by Zacks which he termed a granulocyte. Feng (1965) demonstrated that hemocytes of *Crassostrea virginica* were capable of pinocytosis; Feng, Feng, Burke and Khairallah (1971) also described the cytology, fine structure and cytochemistry of these cells. Martin (1970) studied the cytology and cytochemistry of hemocytes of *Spisula solidissima* and found only one type of cell, a granulocyte. Moore (1972), and Moore, Drake, and Eble (1972) described three types of hemocytes in *M. mercenaria*, a small agranulocyte and two types of granulocytes; they also reported on the cytochemistry of various enzymes, neutral lipids and glycogen. Gurski and Eble (1973) not only described hemocyte *in vitro* behavior but also reported cell counts for *M. mercenaria*. Loy and Eble (1974) described *in vitro* behavior as well as phagocytic aspects of *M. mercenaria* hemocytes as revealed by time-lapse cinematography. Foley and Cheng (1974) published on the cytology and aspects of the cytochemistry of hemocytes of *Mercenaria mercenaria*; they described three types of cells which they termed granulocytes, fibrocytes and hyalinocytes.

Investigation of defense mechanisms in invertebrates, particularly molluscs, was rekindled as a result of Stauber's (1950) pioneering work (Tripp, 1958a, b, 1961; Feng, 1959, 1966a, b; Bang, 1961; Cheng, Thakur and Rifkin, 1969). Hemocytes were found to phagocytose particulate foreign material and pinocytose soluble proteins (Feng, 1965); substances too large to be phagocytosed were surrounded by amoebocytes and encapsulated (Cheng, 1967; Feng, 1967; Cheng and Rifkin, 1970).

Reviews of molluscan hemocytes can be found in Wilbur and Yonge (1966), Cheney (1971) and Narain (1973).

Since there seems to be no general agreement on the various blood cell types in *M. mercenaria*, this paper attempts to delineate their cytology and aspects of their cytochemistry, as well as postulating a definitive classification of these cells. Further, a behavioral analysis of cell types are made based on time-lapse cinematography of cells.

## Materials and Methods

*M. mercenaria* was obtained from Buzzard's Bay, Massachusetts, and Great Bay, New Jersey. Clams were immediately used or maintained up to seven days in a 50 gallon aquarium kept at 22‰ salinity and 16° C.

Blood samples of approximately 0.01 ml were extracted from the ventricle by means of a 1 cc tuberculin syringe fitted with a number 30-gauge needle. Cells were allowed to settle on a cover glass for fifteen minutes in a moist chamber prior to observation or further preparations. Most cytological studies were done on living cells using the Zeiss Photomicroscope II, equipped with phase contrast and Nomarski interference phase optics.

Counts of cell types were made on a hemocytometer, using living cells over the course of one year. Cell types were identified on the basis of cell size and shape, number of cytoplasmic granules and proportion of hyaline cytoplasm, as well as size, position and characteristics of the nucleus. Percentage composition was determined by averaging differential counts of 100 cells using phase contrast optics. Measurements of cells and nuclei were done on living and fixed preparations using a calibrated ocular micrometer.

Hemocytes were supravitally stained by placing two to three drops of 1 : 20,000 concentration of Janus Green B, neutral red, or toluidine blue adjacent to the cells on a cover glass (Humason, 1972; Johnson, 1969); this technique enabled a gradual mixing of the stain and blood fluid which did not disturb the expanded cells. The cover glass was affixed to the slide by a rim of vaseline; this preparation was frequently viewed continuously for many hours without obvious deterioration of cells.

Hemocytes were fixed for two hours in buffered glutaraldehyde solution (3 ml of 50% stock glutaraldehyde, 47 ml of pH 7.5, 0.2 M phosphate buffer) and subsequently stained with Giemsa (1.0 ml of Giemsa stock, 2.0 ml of a 0.2 M phosphate buffer, pH 7.5, 47 ml of distilled water, Humason, 1972) to illustrate cellular morphology.

DNA was stained by the Feulgen reaction (Humason, 1972); hemocytes were fixed in Carnoy's fluid (3:1) or buffered glutaraldehyde. Vena's modification (1967) of the Feulgen reaction was also used.

Table I

*Some morphological characteristics and percentage composition of hemocytes in* Mercenaria mercenaria. *Mean sizes are for living cells. Percentage composition represents averaged differential counts of 100 cells. Approximately thirty animals were sampled (n = 300 of each cell type).*

| Hemocyte type | Cell length (μm) | Nuclear diameter (μm) | Percentage composition |
|---|---|---|---|
| Agranulocyte | $\bar{x}$ = 5.0 | 3.2 | 2.0 |
| | s.d. = 0.68 | 0.55 | 2.1 |
| Small granulocyte | $\bar{x}$ = 28.0 | 5.5 | 61.0 |
| | s.d. = 6.49 | 0.89 | 5.3 |
| Large granulocyte | $\bar{x}$ = 45.0 | 8.5 | 37.0 |
| | s.d. = 7.30 | 1.12 | 6.1 |

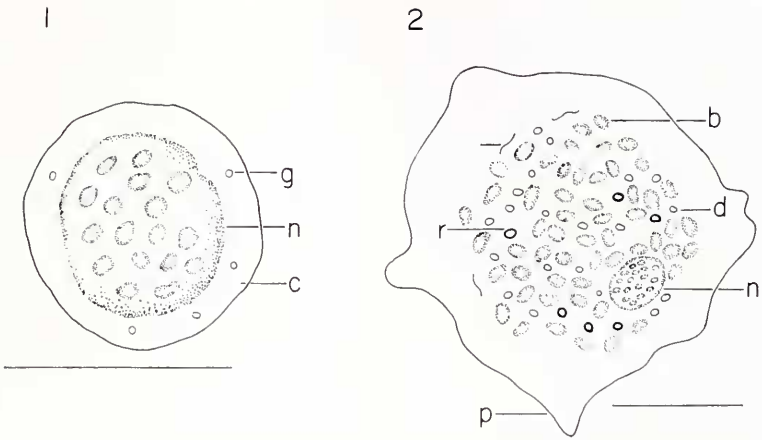



FIGURE 1. Diagram of a "typical" agranulocyte of *Mercenaria mercenaria.* Note the thin rim of cytoplasm surrounding the nucleus and few cytoplasmic granules: c, cytoplasm; n, nucleus; g, granule; 10 $\mu$m bar.

FIGURE 2. Diagram of a "typical" small granulocyte demonstrating four granule types confined to the endoplasm; b, blunt granule; d, dot-like granule; f, filamentous granule; n, nucleus; p, pseudopod; r, refractile granule; 9 $\mu$m bar.

The detection of both lipids and phospholipids employed hemocytes fixed two hours in buffered glutaraldehyde solution; cells were stained with Oil red 0 (Lillie, 1965) or Sudan black B (Humason, 1972), respectively.

Polysaccharides were revealed according to the periodic acid- Schiff (PAS) method of Lillie (1965). Several preparations were incubated 30 minutes in 0.5% alpha amylase solution before oxidation to identify glycogen; cells were pre-

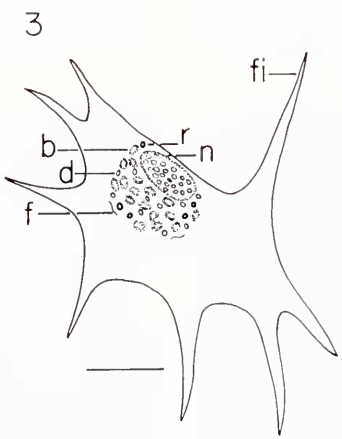
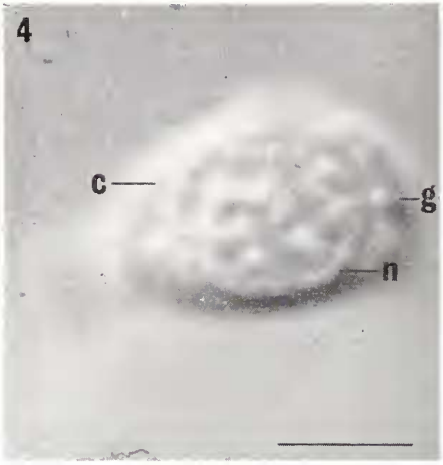



FIGURE 3. Diagram of a "typical" large granulocyte of *Mercenaria mercenaria.* Note the small number of granules as compared with the small granulocyte: b, blunt granule; d, dot-like granule; f, filamentous granule; fi, filopod; n, nucleus; r, refractile granule: 10 $\mu$m bar.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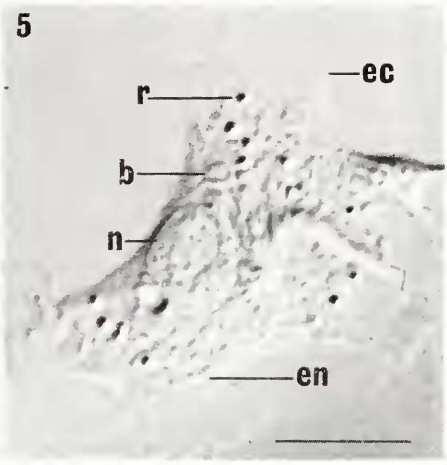
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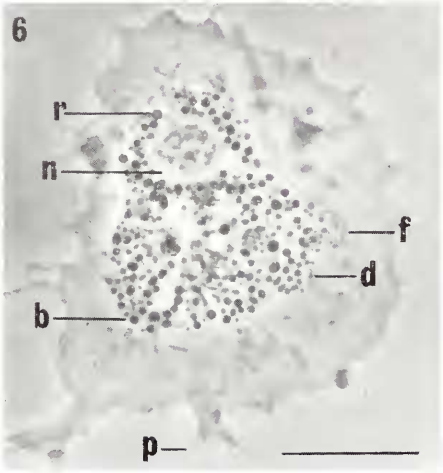
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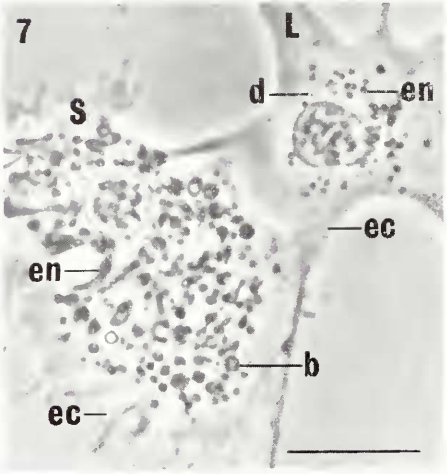
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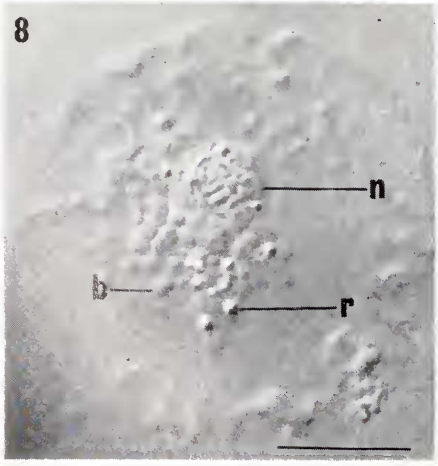
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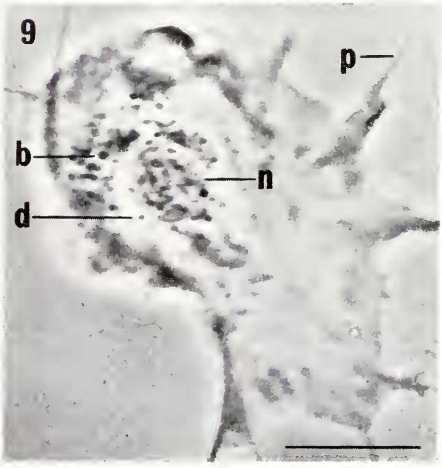
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viously fixed in Davidson's solution (two hours) or buffered glutaraldehyde solution (two hours). The distribution of polysulfated and polycarboxylated polysaccharides were studied in cells stained by the alcian blue pH 5.7 and alcian blue pH 2.6 methods of Mowry (Mowry, 1966; Scott, 1967). Blood cells fixed two hours in buffered glutaraldehyde solution were used in both preparations.

The Fairweather modification of Gomori's procedure was used to detect the presence of lipase activity (Chayen, Bitensky, Butcher and Poulter, 1969). All cells were previously fixed in cold (4° C) buffered glutaraldehyde solution (two hours). Detection of acid phosphatase was accomplished using two different techniques: in the first, cells were prepared according to Burstone's method (1958) after two hours fixation in 4% neutral formalin (4° C); and in the second, cells were fixed two hours in buffered glutaraldehyde solution (4° C) and processed following Goldberg's procedure (Goldberg, 1962; Goldberg and Barka, 1962). Controls were prepared by heat-inactivating cells as well as by omitting substrates from incubation solutions. Novikoff's method (1963) was used to demonstrate the presence of NADH dehydrogenase in cells fixed two hours in buffered glutaraldehyde solution (4° C). Nonspecific esterase was identified using Burstone's method (Pearse, 1962); cells were fixed for two hours in 10% neutral formalin.

Nuclear counterstaining was omitted since all preparations were examined with phase contrast optics which clearly delineated nuclear morphology.

Time-lapse studies were performed with a Zeiss Photomicroscope II fitted with a Sage time-lapse apparatus, Sage Series 500, Sage Instruments Inc., Model A, 16 mm Bolex camera with Plus-X reversal film was used to photograph specimens at an optimum speed of 30–40 frames per minute. Cells were prepared for viewing as for living preparations.

To demonstrate phagocytosis, yeast cells were washed in distilled water and affixed to a cover glass by heating. Hemocytes were placed next to yeast on the cover glass and placed in a moist chamber for 10–15 minutes. Preparations were then fixed in buffered glutaraldehyde solution and stained with Giemsa; some living preparations were also observed.

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FIGURE 4. Agranulocyte as seen under Nomarski interference optics showing an extremely limited amount of cytoplasm and few granules. Note that nuclear morphology is similar to the other two cell types: c, cytoplasm; g, granule; n, nucleus; 3 μm bar.

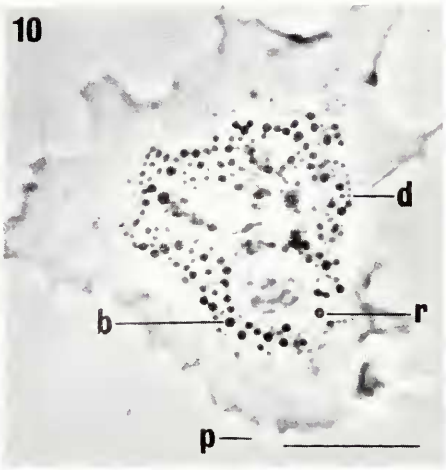
FIGURE 5. Small granulocyte of *Mercenaria mercenaria* viewed with Nomarski interference optics. Note numerous granules and limited ectoplasm: b, blunt granule; ec, ectoplasm; en, endoplasm; n, nucleus; r, refractile granule; 8 μm bar.

FIGURE 6. Small granulocyte supravitally stained with neutral red as seen under phase contrast optics: b, blunt granule, d, dot-like granule; f, filamentous granule; n, nucleus; p, pseudopod; r, refractile granule; 9 μm bar.

FIGURE 7. Large and small granulocytes stained with Janus Green B and viewed with phase contrast optics. Note number of granules present in both granulocytes: b, blunt granule; d, dot-like granule; ec, ectoplasm; en, endoplasm; L, large granulocyte; S, small granulocyte; 10 μm bar.

FIGURE 8. Living large granulocyte as seen under Nomarski interference optics: b, blunt granule; n, nucleus; r, refractile granule; 15 μm bar.

FIGURE 9. Large granulocyte supravitally stained with neutral red and viewed with phase contrast optics. Note uptake of dye by blunt and dot-like granules: b, blunt granule; d, dot-like granule; n, nucleus; p, pseudopod; 15 μm bar.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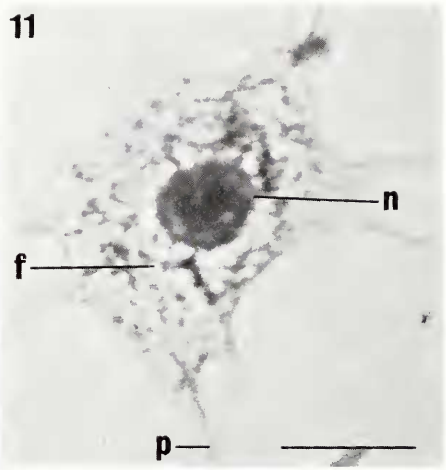
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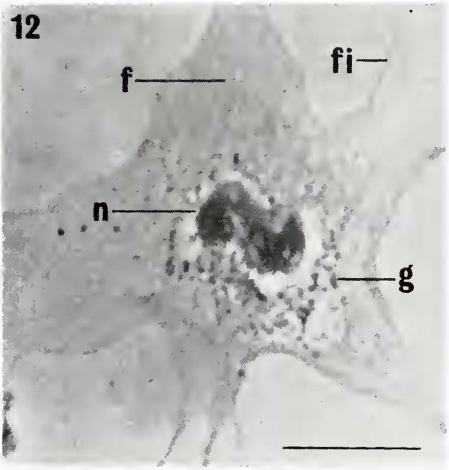
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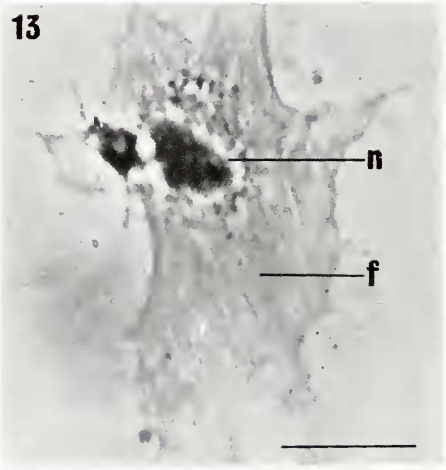
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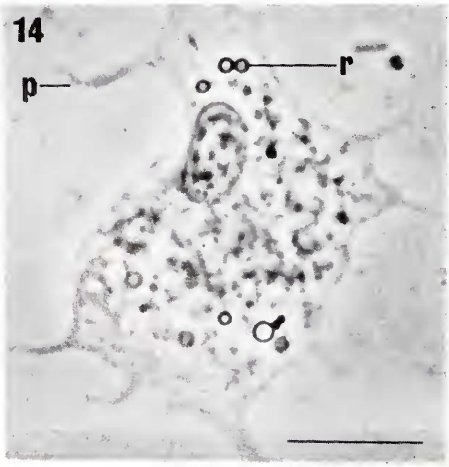
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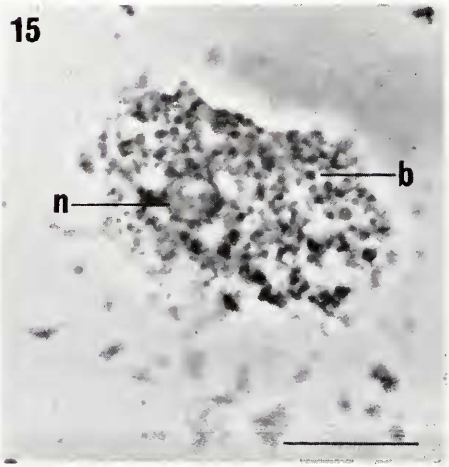
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TABLE II

*Reactions of cytoplasmic granules to cytochemical techniques.*

| Technique | Blunt granule | Dot-like granule | Refractile granule | Filamentous granule |
|---|---|---|---|---|
| Giemsa | acidophilic | acidophilic | acidophilic | acidophilic |
| Janus Green B | + | + | − | − |
| Neutral red | red to amber | red to amber | some red to amber | − |
| Toluidine blue | γ-metachromasia | γ-metachromasia | − | − |
| NADH dehydrogenase | + | − | − | − |
| Acid phosphatase | − | + | − | − |
| Esterase | − | + | − | − |
| Sudan black B | outer boundary positive | entire granule positive | outer boundary positive | − |
| Oil red O | − | − | + | − |
| PAS | + | + | − | − |
| PAS after α amylase | + | + | − | − |
| Alcian blue 2.6 | results were inconclusive | | | |
| Alcian blue 5.7 | results were inconclusive | | | |

## RESULTS

Immediate examination of hemolymph shows blood cells to be rounded to oval. After fifteen minutes in a moist chamber most hemocytes adhere to glass and extend filopods. A detailed study of living and fixed hemocytes reveals three cell types: an agranulocyte and small and large granulocytes (Table I). These hemocyte types are present regardless of the organism's habitat.

### *Cell types*

*Agranulocyte.* This cell averages 5 μm ± 0.68 in diameter with a prominent, centrally situated nucleus surrounded by a thin covering of cytoplasm practically devoid of granules. The rounded nucleus averages 3.2 μm ± 0.55 and has chromatin evenly dispersed throughout with a distinct rim of chromatin lining the nuclear membrane (Figs. 1 and 4). This cell shows no motility and comprises only 2% ± 2.1 of the total cell population.

*Small granulocyte.* The small granulocyte averages 28 μm ± 6.49 in its long

FIGURE 10. Janus Green B-positive granules in a small granulocyte as seen under phase contrast optics: b, blunt granule; d, dot-like granule; r, refractile granule; p, pseudopod; 10 μm bar.

FIGURE 11. Small granulocyte stained with Giemsa. Note fibrous-like material in endoplasm: f, fibrous-like material; n, nucleus; p, pseudopod; 9 μm bar.

FIGURE 12. Giemsa-stained large granulocyte showing fibrous-like material in the ectoplasm: f, fibrous-like material; g, granule; fi, filopod; n, nucleus; 12 μm bar.

FIGURE 13. Giemsa-stained large granulocyte containing double nucleus. Note fibrous-like nature of ectoplasm; f, fibrous-like material; n, nucleus; 12 μm bar.

FIGURE 14. Lipid-filled refractile granules stained by Oil red 0 in a small granulocyte and viewed with phase contrast optics: p, pseudopod; r, refractile granule; 9 μm bar.

FIGURE 15. Localization of NADH dehydrogenase in blunt granules using phase contrast optics: b, blunt granule; n, nucleus; 12 μm bar.

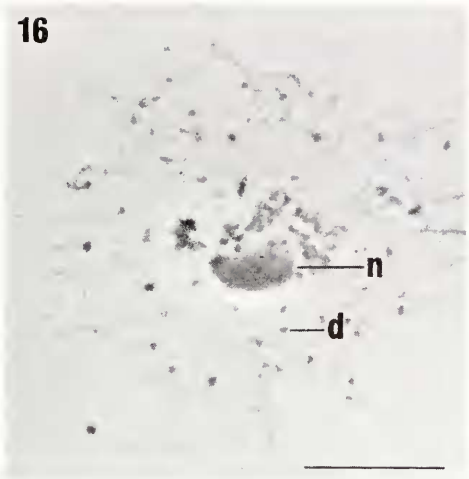
axis. The nucleus is oval, eccentric and averages 5.5 $\mu$m $\pm$ 0.89; nuclear cytology is similar to that of the agranulocytes (Figs. 2 and 5). The cytoplasm contains numerous granules in the endoplasm which can be classified into four types: a blunt, dot-like, filamentous, and refractile granule. Blunt granules, averaging 1.5 $\mu$m in length, are highly plastic (Figs. 6 and 7). These granules are most numerous, representing 52% of all granules. The dot-like granules, with an average diameter of 0.7 $\mu$m, comprise 33% of the granule population. Refractile granules (Figs. 5 and 6) are round with a prominent boundary. They average 1 $\mu$m in diameter and constitute 11% of the total number of granules. Filamentous granules are sparce, making up 4% of all granules and measure 2 $\mu$m in length. The small granulocytes possess a limited amount of clear ectoplasm in which some dot-like granules can be distinguished. This cell type is highly motile and moves in unidirectional patterns. The ectoplasm appears quite ruffled and forms filopods which adhere to the cover glass; occasionally an elongate filopodium forms and terminates in a web-like mass which adheres to the glass. Time-lapse studies show the margins of the small granulocyte to be in a constant state of wavelike motion. Adjacent granulocytes make contact by means of filopods forming a mesh of adhering cells. Granules within the cytoplasm are in constant motion and can be seen flowing from the cell body to the terminal mass. The small granulocyte comprises 61% $\pm$ 5.3 of the hemocyte population.

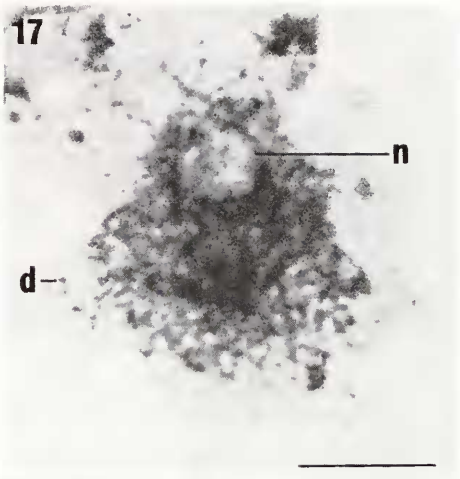
*Large granulocyte.* The large granulocyte averages 45 $\mu$m $\pm$ 7.30 in its long axis (Figs. 3 and 8). While nuclear position and morphology is similar to that of the small granulocyte, it is proportionally larger, averaging 8.5 $\mu$m $\pm$ 1.12 in length. The same four granule types are present in the cytoplasm, again confined to the endoplasm. Granules in large granulocytes are present in the same proportion as in small granulocytes but are only one-third as numerous (Fig. 7). The large granulocyte appears to possess limited mobility under light microscopy. Generally, this cell type spreads on the cover glass by means of filopods which considerably stretches the cytoplasm, giving the cell an extremely thin appearance (Figs. 7 and 9). Time-lapse photomicrography reveals a slow, erratic motion of this cell; the cell membrane and adjacent ectoplasm, however, are similar to the small granulocyte, in a continuous, waving motion. The granules in the endoplasm are also in a highly dynamic, erratic motion. Large granulocytes make up 37% $\pm$ 6.1 of the total cell population.

*Supravital staining*

*Agranulocyte.* No uptake of any vital stain used is observed in agranulocytes.

*Small and large granulocytes.* Blunt and dot-like granules (Fig. 10) of both granulocyte types take up and convert Janus Green B to the red-reduction product, diethyl safranin (Table II) within two hours. When exposed to neutral red, again dot-like and most blunt granules, with an occasional refractile granule, take up the dye in both small (Fig. 6) and large (Fig. 9) granulocytes. A color change from red to amber occurs from 30 seconds up to several hours, generally being slower in the larger granulocytes. Supravital studies with toluidine blue show similar metachromatic reactions in both granulocyte types (Table II), the large granulocytes again reacting slower.



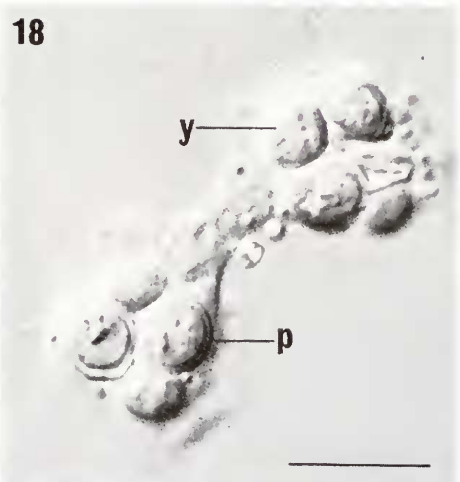




FIGURE 16. Acid phosphatase activity in dot-like granules as seen under phase contrast optics: d, dot-like granule; n, nucleus; 15 μm bar.

FIGURE 17. Nonspecific esterase activity in dot-like granules of small granulocyte viewed with phase contrast optics: d, dot-like granule; n, nucleus; 10 μm bar.

FIGURE 18. Yeast cells phagocytozed by a large granulocyte as seen with Nomarski interference optics: p, phagosome; y, yeast; 10 μm bar.

*Cytochemistry*

*Giemsa.* Observations of Giemsa preparations reveal the nucleus to stain basophilic, while cytoplasmic granules appear pink to red in both small (Fig. 11) and large (Figs 12 and 13) granulocytes. The agranulocyte was not observed.

*Feulgen staining.* All three hemocyte types show a similar nuclear morphology after staining with Feulgen. Dense areas of chromatin, uniformly distributed, stain deep violet. A dark staining rim of chromatin lines the nuclear membrane.

*Lipids and phospholipids.* Only refractile granules produce a lipid-positive

reaction when stained with Oil red 0 (Fig. 14). Sudan black B is preferentially taken up by the outer boundary of the nucleus as well as the membranes of refractile and blunt granules in small and large granulocytes. Dot-like granules appear as solid black specks within the endoplasm and ectoplasm. Neither lipid nor phospholipid staining is seen in agranulocytes.

*Polysaccharides.* Periodic acid-Schiff reaction produces deep-red staining of dot-like and most blunt granules in small and large granulocytes. After treatment with δ-amylase, there is no reduction in staining intensity. Agranulocytes do not stain in this preparation. The detection of polysulfated and polycarboxylated polysaccharides proved inconclusive.

*Enzymes.* NADH dehydrogenase activity is localized in blunt granules (Fig. 15) of both small and large granulocytes. Agranulocytes appear to exhibit some activity, but this activity could not be identified in a distinct granule type. Acid phosphatase-positive granules are highly irregular with respect to shape and size (Fig. 16). Large and small granulocytes show 20–30 scattered granules, measuring slightly larger than dot-like but smaller than blunt granules. Nonspecific esterase staining of dot-like granules (Fig. 17) is observed in both granulocyte types. While the number of dye granules of acid phosphatase corresponds to the number of dot-like granules identified in living preparations, the number of dye granules of nonspecific esterase are much more numerous. Agranulocytes also demonstrate minute centers of nonspecific esterase activity in their limited cytoplasm.

## Discussion

Hemocytes of *Mercenaria mercenaria* may be divided into three types: agranulocytes, small granulocytes and large granulocytes. This classification is based on the following criteria: cell size, nuclear size, number of cytoplasmic granules, the ratio between endoplasm and ectoplasm, and motility.

The agranulocyte, with limited cytoplasm, has been identified by other investigators (Cuénot, 1891; Drew and Cantab, 1910; George and Ferguson, 1950; Dundee, 1953) in examining a variety of organisms. The original work on *Mercenaria mercenaria* hemocytes by Zacks (1955) describes only one cell type, a granulocyte. Later work by Foley and Cheng (1974) identified three classes of hemocytes but subsequently reduced this number to two (Cheng and Foley, 1975). The latter authors base their classification system on the presence of granules, as well as the possession of filopodia containing cytoplasmic fibers. Our studies have shown the large granulocyte capable of forming and absorbing filopodia containing cytoplasmic fibers in *in vitro* preparations. Hence, the fibrocyte and hyalinocyte types of Foley and Cheng (1974) appear to correspond to our large granulocyte. The cell we have classified as a small granulocyte is also referred to as a granulocyte by Foley and Cheng (1974).

Differential motility noted among hemocytes was also observed by Foley and Cheng (1974). The extreme motility of small granulocytes is due to a constant waving of ectoplasmic borders and advancing filopodia. Foley and Cheng (1974) describe a similar motility pattern for hemocytes designated as "granulocytes". Time-lapse studies (Loy and Eble, 1974) show that large granulocytes move by

very slow, sliding motions with much waving of ectoplasmic borders; this appears to correspond to the motility of "agranulocytes" as explained by Foley and Cheng (1974).

The four granule types identified (blunt, dot-like, refractile and filamentous) exhibited specific reactions to the cytochemical techniques used (Table II). In Giemsa preparations the cytoplasm stains acidophilic, while the nucleus stains basophilic (Figs. 11 and 13). This was previously reported in *Mercenaria mercenaria* by Zacks (1955). The intense pink staining of cytoplasm and granules is contrary to findings by Foley and Cheng (1974), who report a network of blue inclusions and pale blue cytoplasm. Although individual granules are noted by Zacks (1955) and further delineated by Foley and Cheng (1974), no comprehensive study of granule types has been published.

Based on the ability of blunt granules to reduce Janus Green B to diethyl safranin, localization of NADH dehydrogenase activity in them (Fig. 15), their extreme plasticity (Lehninger, 1964), and boundary staining with Sudan black B, it can be concluded that these granules are mitochondria. Zacks (1955) designed "specific granules" as atypical mitochondria as a result of positive reactions to these cytochemical methods. Foley and Cheng (1974) found that nearly all cytoplasmic granules, with the exception of refractile inclusions, take up both Janus Green B and neutral red.

Localization of acid phosphatase produces very irregular but discrete centers of activity (Fig. 16). The fact that the granules measure slightly larger than the mean size of dot-like granules is attributed to possible accretion of dye due to the long incubation time (18 hours). Dot-like granules (Fig. 17) are also identified as centers of nonspecific esterase activity (Moore, Drake, and Eble, 1972). Zacks and Welsh (1953) found no esterase activity in *M. mercenaria* but indicated their histochemical experiments were not conclusive. Centers of nonspecific esterase activity are more numerous than those of acid phosphatase, indicating that many of these granules are very small or more permeable to reagents and not usually visible. The presence of nonspecific esterase in addition to acid phosphatase, which has been considered a marker for lysosomes (deDuve, 1963) suggests the lysosomal function of dot-like granules (Feng, Feng, Burke and Khairallah, 1971; Holt, 1963). Yoshino and Cheng (1976) have also identified centers of acid phosphatase activity in *Mercenaria mercenaria* as lysosomes. Supravital staining of dot-like granules by neutral red, thought to be indicative of lysosomes (Byrne, 1964a, b; Humason, 1972) also points to a lysosomal physiology. In addition, the uptake of Sudan black B demonstrates the presence of phospholipids, possibly as a membrane structure, typical of lysosomes (deDuve, 1963; Tappel, Sawant and Shibko, 1963).

Refractile granules demonstrate a positive reaction for lipids (Fig. 14), when stained with Oil red 0. The outer boundary staining of these granules with Sudan black B demonstrates the presence of phospholipids. On the basis of these reactions, it appears that refractile granules function as lipid storage centers.

The function of filamentous granules observed in living preparations is not clear since they are not seen to react with any of the cytochemical techniques. "Vermiform bodies" described by Foley and Cheng (1974) might correspond to this granule type.

Incorporation of bacteria into cytoplasmic vacuoles is occasionally observed in small and large granulocytes but never in agranulocytes. This suggests a phagocytic function for both granulocyte types of *M. mercenaria*. Work by Gurski and Eble (1973) shows small and large granulocytes phagocytizing yeast (Fig. 18) and three species of bacteria. Foley and Cheng (1975) have also found that hemocytes of *Mercenaria mercenaria* will phagocytize bacteria. Gurski and Eble (1973) demonstrated phagocytosis of yeast to be a direct function of yeast concentration. They further found small granulocytes to have a higher phagocytic rate than large granulocytes. Time-lapse cinematography (Loy and Eble, 1974) reveals phagocytosis in small granulocytes to occur by rapid cell extensions in and around the yeast, until the latter are incorporated within the cell as phagosomes. Large granulocytes however, phagocytose yeast cells by enveloping them with wave-like extensions of the outer ectoplasm.

It has been proposed by several authors (Cuénot, 1891; Drew and Cantab, 1910; Haughton, 1934; George and Ferguson, 1950) that various types of invertebrate hemocytes represent stages in the life cycle of one cell type. The high degree of nuclear morphological similarity in all three hemocytes of *M. mercenaria* supports this concept. Evidence is also offered by the presence of identical granule types in both small and large granulocytes. Furthermore, granules of granulocytes demonstrate similar reactions to all staining techniques. Based on these findings, it might be postulated that the agranulocyte represents the immature stage which gives rise to the small motile granulocyte upon formation of additional cytoplasm and granules; the large granulocyte may be the degenerate phase of the cycle with the cell gradually losing its motility as it undergoes degranulation. It is consistently observed that the granules of the large granulocyte take up supravital stains at a slower rate than corresponding granules of the small granulocyte. The fact that Loy and Eble (1974) found the large granulocyte to be a less active phagocyte than the small granulocyte also lends support to this hypothesis. Feng, Feng, Burke and Khairallah (1971) have described a senile stage in the hemocytes of *Crassostrea*, but nothing of this nature has been observed in *Mercenaria mercenaria*. Further work is required to determine whether the cell types described here are different development stages of a single cell line or if they are indeed separate and distinct cell lines.

## Summary

The hemocytes of the hard clam *M. mercenaria* were of three types: an agranulocyte, a small, and a large granulocyte. The agranulocyte, with only a thin periphery of cytoplasm surrounding the nucleus, had no visible cytoplasmic granules in living preparations but did exhibit a few centers of nonspecific esterase activity. This cell type represented 2% of the hemocyte population. The small granulocyte possessed four distinct granule types and comprised 61% of the total cell population. Large granulocytes accounted for 37% of all hemocytes. While they contained the same four granule types identified in the small granulocyte, only one-third the total number were present. The nucleus of all three hemocyte types appeared morphologically similar.

The four types of granules observed were a blunt, dot-like, a refractile and a filamentous granule. Blunt granules were identified as mitochondria, based on

their ability to reduce Janus Green B to diethyl safranin, the presence of NADH dehydrogenase activity and boundary staining with Sudan black B. Dot-like granules were identified as lysosomes on the basis of neutral red staining, localization of acid phosphatase and nonspecific esterase activity and staining with Sudan black B. Refractile granules were demonstrated to be membrane-bound, lipid-filled structures that reacted positively with Sudan black B and Oil red 0, respectively; these granules act as lipid storage centers.

Nuclear similarity of the three cell types suggest that these cells might represent different stages of maturity, rather than three distinct cell lines. This was also indicated by the similar yet graded cytochemical reactions and the varying degree of motility and phagocytic activity demonstrated by hemocyte types.

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